



## Characterization of Biosurfactants from Endophytic Bacteria Isolated from Geothermal Fern Leaves D9

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### Abstract

Biosurfactants are surface-active compounds synthesized by microorganisms. These compounds offer several advantages, including low toxicity, high compatibility, and biodegradability. Biosurfactants can be isolated relatively quickly, a property that renders them potentially applicable in industrial settings. One type of microorganism that produces biosurfactants is endophytic bacteria, which live within plant tissues. The present study focuses on the characterization of biosurfactants produced by endophytic bacteria isolated from geothermal ferns in the area around the Gedong Songo hot spring in Semarang Regency. Isolate 9 (D9) demonstrated the optimal oil-spreading capability and was consequently selected for further production and characterization. The optimal growth curve for biosurfactant production by isolate D9 (BS-D9) occurred in the early stationary phase, approximately 132 hours post-inoculation, with an oil-spreading diameter of 5.1 centimeters. The emulsification index (E24) of BS-D9 reached 95%, which is nearly equivalent to SDS at 1000 ppm, suggesting a high emulsification capacity. The methylene blue test indicated that BS-D9 is an anionic biosurfactant, whereas the negative Biuret test result indicated that it does not belong to the lipopeptide group. Fourier-transform infrared (FTIR) spectroscopy indicates that BS-D9 falls within the glycolipid category. This classification is supported by a distinctive absorption peak corresponding to glycosidic bonds, potentially of the rhamnolipid type. Rhamnolipids are widely recognized for their high emulsification efficiency, stability, and environmental compatibility, making them attractive for various industrial applications. These results indicate that BS-D9 has potential applications as a surface-active agent with environmentally friendly properties.

### 1. Introduction

The cosmetics and pharmaceutical industries are experiencing global growth, prompting exploration of bioactive compounds to develop more innovative product formulations. The incorporation of natural products is a significant trend in health-oriented formulations [1]. One of the natural products utilized is lecithin surfactant from soybeans, which functions as an emulsion stabilizer. While the efficacy of the lecithin surfactant is well documented, the isolation process is costly and time-consuming. Lecithin extracted from soybean oil has a yield of 1.55% [2]. This condition has prompted the search for more economical and environmentally friendly

surfactant alternatives, one of which is biosurfactants from microorganisms [3].

Microorganism biosurfactants hold considerable promise for application in the cosmetics and pharmaceutical industries, as they exhibit greater biological safety than surfactants derived from natural ingredients. The following characteristics are of particular importance in determining the potential of biosurfactants for commercial applications in both industries: antimicrobial activity, antioxidant properties, the ability to enhance skin penetration, and stability under diverse pH and temperature conditions [4]. Research on biosurfactants in the cosmetic and pharmaceutical fields primarily focuses on the potential

of microorganism-derived compounds. These compounds can be classified into several categories, including glycolipids [5, 6], lipopeptides and lipoproteins [7, 8], phospholipids, and fatty acids [9]. For instance, *Pseudomonas aeruginosa*, a bacterium capable of producing rhamnolipids, has demonstrated the capacity to exhibit moisturizing, antibacterial, and drug delivery agent properties [10].

Another alternative microorganism with the potential to produce biosurfactants is endophytes, microbes that live within plant tissues without harming their hosts. Host plants have been observed to absorb hydrocarbons from the soil and transport them to areas where endophytic bacteria are located. The production of biosurfactants by endophytic bacteria has been demonstrated to facilitate biofilm formation and cell signaling, thereby potentiating interactions between plants and endophytes [11]. Munakata *et al.* [12] indicates that endophytic bacteria isolated from fragrant roots have the capacity to produce lipopeptide biosurfactants. Chopra *et al.* [13] on *Pseudomonas aeruginosa* RTE4 isolated from tea, has demonstrated its capacity to produce rhamnolipid biosurfactants, while A. *et al.* [14] on endophytic *Burkholderia sp.* WYAT7, an endophytic bacterium, has shown its ability to produce glycolipid biosurfactants.

Endophytic bacteria isolated from plants growing in extreme environments have been reported to possess potent active compounds. Geothermal areas are characterized by elevated temperatures and low pH, creating extreme physical and chemical conditions that pose significant challenges for plant growth. The capacity of organisms to thrive in extreme environments is facilitated by the presence of endophytic microorganisms. The presence of biosurfactants has been shown to facilitate microbial access to nutrients by reducing surface tension and emulsifying hydrophobic compounds [15]. Furthermore, biosurfactants have been shown to protect cells from environmental stress by maintaining membrane integrity and facilitating the formation of protective biofilms [16]. In geothermal environments, biosurfactants have been observed to enhance the solubility of metals from hot sediments by interacting with water at mineral surfaces [17].

This phenomenon prompted the Biotransformation Research Team of the Department of Chemistry at Diponegoro University to isolate endophytic bacteria from ferns growing in the area around the Gedong Songo hot spring. Previous research has successfully isolated 16 endophytic bacteria isolates from ferns (unpublished). Oil dispersion screening revealed that all isolates produced biosurfactants, though not all in significant quantities. Isolate D9, in particular, demonstrated a notable capacity for biosurfactant production, exhibiting high levels of this specialized substance. The objective of this study is to further explore the production, purification, and characterization of biosurfactants from isolate D9.

## 2. Experimental

### 2.1. Bacterial Rejuvenation

The bacterial rejuvenation process was implemented to obtain more productive isolates. Liquid media in the form of nutrient broth were prepared and subsequently sterilized in an autoclave at 121°C for 45 minutes. Following the sterilization process, bacterial samples from the glycerol stock collection (obtained from the Biotransformation Research Team at the Department of Chemistry, Diponegoro University) were inoculated into the liquid medium and incubated for 24 hours at 37°C. Turbidity in the liquid medium indicated bacterial growth. Slant agar was prepared by thoroughly dissolving nutrient broth and agar in distilled water within an Erlenmeyer flask. The mixture was sterilized in an autoclave at 121°C for 50 minutes, then transferred to test tubes and left to solidify. Subsequently, endophytic bacteria obtained from the liquid medium were inoculated onto slant agar and incubated for 24 hours at 37°C.

### 2.2. Starter Preparation

The bacterial stock from the slant agar was then inoculated into sterile nutrient broth (NB). The bacterial stock density was subsequently measured using a UV-Vis spectrophotometer at 600 nm. Optical density (OD) measurements were performed to obtain a bacterial density corresponding to the 0.5 McFarland standard, which is in the OD range of 0.08–0.20. This 0.5 McFarland standard is equivalent to a bacterial concentration of approximately  $1.5 \times 10^8$  CFU/mL.

### 2.3. Growth Curve Construction and Oil Spreading Assay

Growth curves were developed to ascertain the optimal harvest time for biosurfactants. The experimental design encompassed assessments of bacterial cell proliferation and the efficacy of biosurfactants in oil dispersion. The biosurfactant growth medium was prepared according to the study's guidelines [18]. It contained a mineral composition of 0.075 g of  $\text{NH}_4\text{NO}_3$ , 0.6 g of NaCl, 0.075 g of  $\text{KH}_2\text{PO}_4$ , 0.075 g of  $\text{K}_2\text{HPO}_4$ , and 0.075 g of  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , dissolved in 150 mL of distilled water. The pH was adjusted to 7. A carbon source composed of 3% (v/v) palm oil and 1% (v/v) of the endophytic bacterial starter isolate D9 was added to the medium, as palm oil provides a hydrophobic, fatty-acid-rich substrate that effectively induces biosurfactant production. The mixture was then incubated in a rotary shaker at 37°C. Bacterial cell growth was measured by turbidimetry using a UV-Vis spectrophotometer at 600 nm. Given that palm oil in the medium may contribute to light scattering and affect absolute optical density values, OD measurements were primarily used to monitor relative growth trends and determine growth phases rather than to quantify absolute cell biomass. Biosurfactant activity was assessed using an oil-spreading assay with measurements every 12 hours.

### 2.4. Biosurfactant Production

The biosurfactant production process was carried out according to the optimal conditions determined from the growth curve. The growth medium employed a mineral medium composition and carbon sources consistent with those utilized in the growth curve production. Incubation was conducted at 37°C in a shaker incubator under dark conditions. These dark conditions were applied to maintain stable incubation parameters and minimize potential physicochemical changes or degradation caused by light exposure during fermentation. The fermentation process was halted at the point where maximum biosurfactant production was attained. Subsequently, a centrifugation process was initiated to separate the cell biomass from the upper layer of the mixture. The resulting material was then subjected to additional processing.

### 2.5. Purification of Biosurfactants

The supernatant was adjusted to pH 2 with HCl and incubated at 4°C for 12 hours. The solution was extracted with a mixture of chloroform and methanol at a volume ratio of 2:1 (v/v), with a supernatant: chloroform: methanol ratio of 1:2:1. The drying process was carried out using a rotary evaporator. The dry biosurfactant extract was stored for subsequent analysis [14].

### 2.6. Oil Spread Assay

The oil dispersion test was conducted by pouring 30 milliliters of distilled water into a petri dish, then adding 1 milliliter of used oil to the surface of the water, forming a thin layer. Distilled water was utilized as a negative control, while sodium dodecyl sulfate (SDS) was employed as a positive control. In the experimental setting, both BS-D9 and SDS were evaluated at a concentration of 1,000 parts per million (ppm). This evaluation was conducted by applying 20 µL of the solution to the surface of the oil layer. The degree of biosurfactant activity was determined based on the diameter of the clear zone formed on the oil layer [14].

### 2.7. Determination of Emulsification Index (E24)

The emulsion test was conducted using palm oil at a 1:1 volume ratio with the test sample. Distilled water was utilized as a negative control, while SDS was employed as a positive control. The substances known as BS-D9 and SDS were meticulously prepared at concentrations of 1,000 ppm, 100 ppm, 10 ppm, and 1 ppm, respectively. Each solution was then mixed with palm oil in a test tube at a 1:1 ratio and subsequently homogenized using a vortex. The mixture was then left at room temperature for 24 hours. The height of the emulsion layer formed was measured, and the emulsion index value was subsequently calculated using Equation (1) [14].

$$\%E24 \text{ index} = \frac{\text{Height of emulsion}}{\text{Total height (emulsion+oil)}} \times 100 \quad (1)$$

### 2.8. Methylene Blue Active Substances (MBAS) Assay

The methylene blue test was conducted to determine the anionic properties of biosurfactants. Distilled water served as the negative control, while SDS was used as the positive control. BS-D9 and SDS samples were prepared

at 1,000 ppm. A total of 1 mL of test solution was mixed with 1 mL of 0.01% methylene blue solution and 1 mL of chloroform in a test tube. The mixture was subsequently homogenized, after which it underwent phase separation. Anionic biosurfactants are indicated by a blue color shift to the chloroform phase [19].

### 2.9. Biuret Test

The Biuret test was performed to detect peptide bonds in BS-D9. The negative control consisted of distilled water, while the positive control used bovine serum albumin (BSA). The BS-D9 and BSA samples were prepared at a concentration of 1,000 ppm. A total of 2 milliliters of test solution was added to 1 milliliter of a 10% NaOH solution, as well as several drops of a 1% CuSO<sub>4</sub> solution. The mixture was subjected to gentle agitation, after which a color change was discerned. The presence of a purple coloration is indicative of the presence of peptides within the sample [20].

### 2.10. Fourier Transform Infrared Spectroscopy (FTIR) Analysis

The analysis, facilitated by Fourier Transform Infrared Spectroscopy (FTIR), aims to identify the predominant functional groups of biosurfactants produced by endophytic bacteria inhabiting ferns. The samples were initially compressed using a hydraulic apparatus to form solid pellets that were suitable for spectroscopic analysis. The pellets were then analyzed using FTIR in transmission mode. The analysis was performed at a resolution of 4 cm<sup>-1</sup> in the wavenumber range between 400 cm<sup>-1</sup> and 4000 cm<sup>-1</sup> to identify functional groups and molecular interactions in the sample [21].

## 3. Results and Discussion

### 3.1. Growth Profile of Isolate D9 and Biosurfactant Production

The objective of this study was to determine the growth phase of endophytic bacterial isolate D9 and to identify the optimal production time of its biosurfactant. Observations were made by measuring the optical density of bacterial growth and the diameter of oil dispersion at 12-hour intervals. The results are shown in Figure 1.

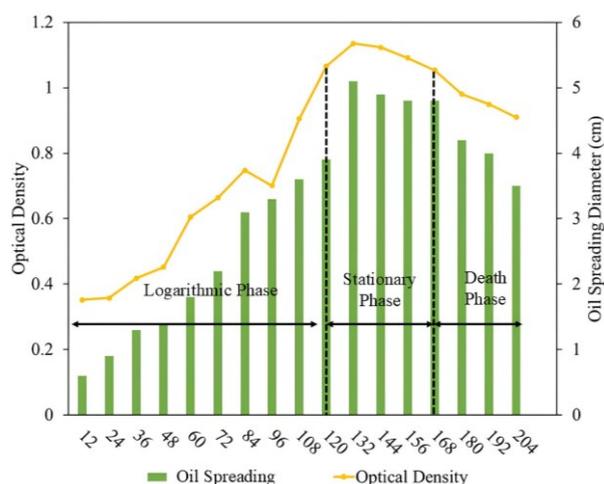


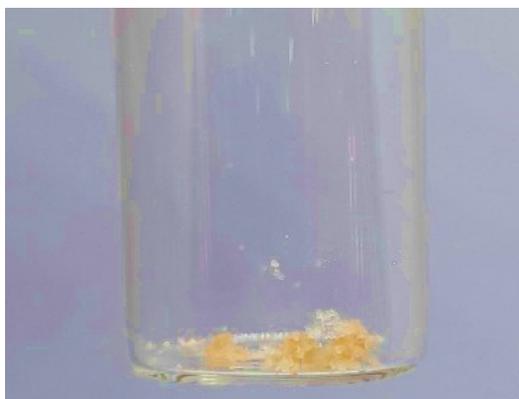
Figure 1. Growth curve of endophytic bacteria isolate D9

The logarithmic phase was observed from hour 12 to hour 120. The stationary phase commenced at hour 132 and persisted until hour 168. The duration of the death phase was documented from hour 180 to hour 204. The relatively prolonged logarithmic phase observed in this study is likely associated with the use of palm oil as a complex and hydrophobic carbon source, which requires gradual adaptation and enzymatic processing before efficient cellular utilization. As illustrated in Figure 1, the results of the bacterial growth curve analysis demonstrate that biosurfactant production commences at the onset of the stationary phase, specifically at 132 hours, when the OD was 1.121, and the oil spreading diameter was 5.1 cm.

As stated by Varjani and Upasani [22], biosurfactants are secondary metabolites produced during the stationary phase of microbial growth. From a genetic perspective, biosurfactant production can occur even in the presence of ample nutrients, yet bacteria are unable to directly utilize hydrophobic carbon sources, such as oil. Consequently, biosurfactants are secreted to emulsify hydrophobic substrates, thereby facilitating their dispersion in the aqueous phase and enhancing accessibility for cells. The increase in biosurfactant production is directly proportional to emulsion formation, which in turn supports cell population growth. However, in the late stationary phase, the concentration of biosurfactants decreases because these compounds, such as glycolipids, can be reused as an energy source by bacteria [23]. Biosurfactant production is also regulated by genetic mechanisms. For instance, in *Pseudomonas aeruginosa*, the expression of the *rhlAB* gene, which encodes rhamnolipid biosynthesis, is subject to regulation by the quorum-sensing system. This system is only activated when growth is arrested due to nitrogen limitation, despite the presence of ample carbon [24].

### 3.2. Production and Purification of Biosurfactants

The biosurfactant produced at 132 hours was separated from the biomass and the supernatant by centrifugation. Biosurfactant purification has been achieved using methanol and chloroform extraction methods. The biosurfactant in the polar phase was evaporated, yielding a dry extract of biosurfactant isolate D9 (BS-D9) that was yellow in color, as shown in Figure 2.



**Figure 2.** Dried extract of biosurfactant isolate D9 (BS-D9)

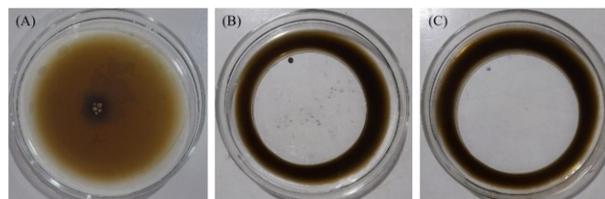
The biosurfactant produced by *Nesterenkonia* sp. is a lipopeptide that manifests as a yellow solid [25]. Qin *et al.* [26] indicates that the biosurfactant produced from *Bacillus subtilis*, a lipopeptide surfactin, manifests as a yellowish solid. Rhamnolipid biosurfactants, produced by *Pseudomonas aeruginosa*, are characterized by their ability to generate viscous, dense, and brownish-yellow biosurfactants [27]. Sultana *et al.* [28] reported that rhamnolipid biosurfactants produced by *Bacillus velezensis* S2 result in the formation of thick, brown compound.

### 3.3. Oil Spread Assay

The oil spreading assay is a method used to assess the efficacy of biosurfactants in distributing oil on the water surface. The oil spreading assay for BS-D9 and SDS at 1000 ppm yielded values of  $5.1 \pm 0.10$  cm and  $5.5 \pm 0.10$  cm, respectively (Figure 3). The oil-dispersing propensity of biosurfactants depends on their inherent hydrophobic and hydrophilic characteristics. The hydrophilic head integrates into the aqueous phase and interacts with water molecules, while the hydrophobic tail allows it to partition into the oil phase. This interaction disrupts the cohesion between oil molecules, thereby facilitating their spreading [29].

The oil spread diameter measured by BS-D9 appears to be equivalent to that by SDS in Figure 3, indicating that BS-D9 has a strong oil-spreading capacity. Liu *et al.* [30] reported that the biosurfactant produced by strain S2 from oil field wastewater isolates showed an oil spreading diameter of 14.0 cm, which was significantly higher than the diameters of strains S3 (8.5 cm) and S6 (7.6 cm). This finding suggests that strain S2 exhibits enhanced biosurfactant production potential, characterized by elevated biosurfactant levels and improved properties.

Research by Jiang *et al.* [31] reported that biosurfactants produced by bacteria isolated from marine sediments had oil-spreading diameters of 8.2 cm (N33), 7.9 cm (Nian2), and 5.0 cm (T10). Albasri *et al.* [32] demonstrated that the oil spreading diameter of rhamnolipids isolated from HA-2 was 5.2 cm. However, these studies did not use synthetic surfactants, such as SDS, as positive controls, thereby precluding an accurate comparison of the reported oil-spreading capacity of biosurfactants with that of synthetic surfactants. The disparities in oil spreading diameter can be attributed to variations in microorganism type, medium composition, culture conditions, and biosurfactant extraction methods.



**Figure 3.** Oil spread assay: (A) Negative control (distilled water), (B) BS-D9 (1000 ppm), (C) Positive control (SDS, 1000 ppm)

### 3.4. Determination of Emulsification index (E24)

Emulsification tests were conducted to determine the ability of biosurfactants to form emulsions between two immiscible phases. The emulsification capacity of biosurfactants is quantified by measuring emulsification index values. SDS was used as a positive control, and distilled water as a negative control. A series of concentrated SDS and BS-D9 solutions was meticulously prepared. The results of biosurfactant emulsification are shown in Figure 4.

The findings indicate that BS-D9 has an emulsion index (E24) nearly equivalent to SDS, the positive control. It has been demonstrated that BS-D9 at 1,000 ppm exhibits an emulsion index (E24) of 95%. The emulsification test corroborates the outcomes of the oil dispersion test, in which SDS has been shown to possess superior oil-dispersion and emulsification capacity. Compared with other studies, this study has achieved higher results. As reported in a study of Liu *et al.* [30], the biosurfactants produced by strain S2 from oil field wastewater isolates exhibited an E24 value of 83.0%. Albasri *et al.* [32] reported an E24 of 52% for rhamnolipid biosurfactants produced by *Geobacillus stearothermophilus*. Similarly, A. *et al.* [14] documented that glycolipid biosurfactants derived from *Burkholderia* sp. WYAT7 demonstrated an E24 value of 60%.

A high emulsification index (E24) indicates biosurfactant efficacy, reflecting the stability of the emulsion formed during incubation. In glycolipid biosurfactants, effective emulsification is strongly influenced by the presence of a hydrophilic carbohydrate head group and hydrophobic fatty acid chains. The FTIR spectra of BS-D9 showing strong hydroxyl (–OH) bands associated with carbohydrate moieties and prominent aliphatic C–H stretching vibrations indicate the presence of a polar sugar head group coupled with relatively long hydrophobic fatty acid chains. This balanced amphiphilic structure promotes strong interfacial adsorption and enhances emulsion stability, explaining the high E24 value (95%). Biosurfactants with such structural characteristics are highly promising for applications in cosmetics and pharmaceutical industries [33].

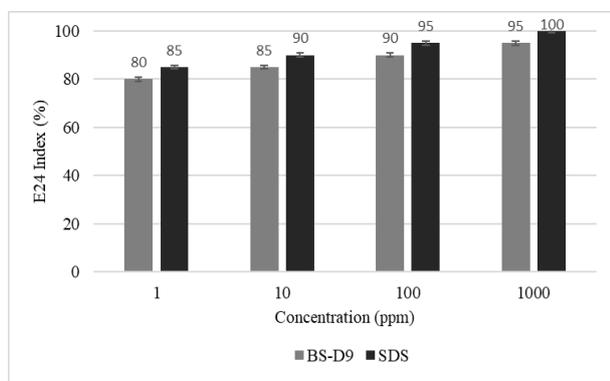


Figure 4. Emulsification Index (E24) result

### 3.5. Methylene Blue Active Substances (MBAS) Assay and Biuret Test

The objective of the methylene blue test is to ascertain whether BS-D9 exhibits anionic or cationic characteristics. Methylene blue is a cationic dye with a positive charge. It has been demonstrated that methylene blue binds ionically to negatively charged biosurfactants, also known as anionic surfactants. When anionic biosurfactants are mixed with methylene blue in a system of water and organic solvents (such as chloroform), an ionic complex is formed that is soluble in the organic phase. A favorable outcome is indicated by the presence of a blue color in the chloroform phase at the base.

As demonstrated in Figure 5(A), isolate BS-D9 exhibited anionic biosurfactant characteristics, as indicated by the formation of a blue color in the chloroform phase. This finding aligns with prior reports [34] that biosurfactants produced by *Kocuria marina* BS-15, *Kurthia* sp. (Ku), *Coprococcus* sp. (Cc), and *Halococcus* sp. (Hc) exhibited favorable outcomes in the methylene blue test, suggesting anionic properties. Furthermore, biosurfactants produced by *Burkholderia* sp. WYAT7 has been reported to exhibit analogous characteristics [14]. Anionic biosurfactants possess negatively charged polar groups, including carboxylate (–COO–) and sulfate (–SO<sub>4</sub><sup>-</sup>) groups. Anionic groups interact electrostatically with methylene blue, forming ion pairs. Ion pair complexes are characterized by their lipophilic nature, which leads to their propensity to dissolve in chloroform. This reaction leads to a decrease in the intensity of the blue color in the aqueous phase [19].

The Biuret test is a method used to detect peptide bonds in biosurfactants. The basis of this test is the interaction of Cu<sup>2+</sup> ions (copper II) in a basic solution (typically NaOH) with peptide bonds (–CO–NH–) present within the sample. A solution containing peptides is then mixed with a biuret solution (copper sulfate and strong base), resulting in the formation of a purple-colored complex with the peptide group [35].

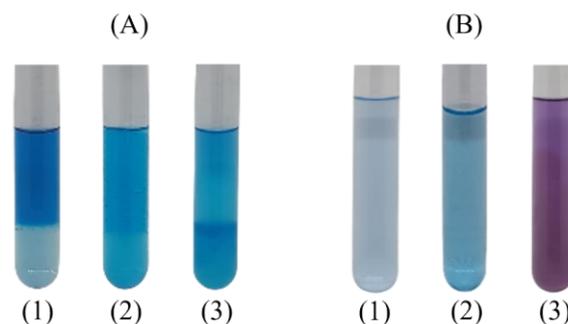


Figure 5. Chemical characterization of BS-D9 biosurfactant (A) MBAS assay: (1) Negative control (distilled water), (2) BS-D9 (1000 ppm), (3) Positive control (SDS, 1000 ppm). (B) Biuret test: (1) Negative control (distilled water), (2) BS-D9 (1000 ppm), (3) Positive control (BSA, 1000 ppm)

**Table 1.** Results of the identification of glycolipid biosurfactant groups produced by several endophytic bacteria in different studies

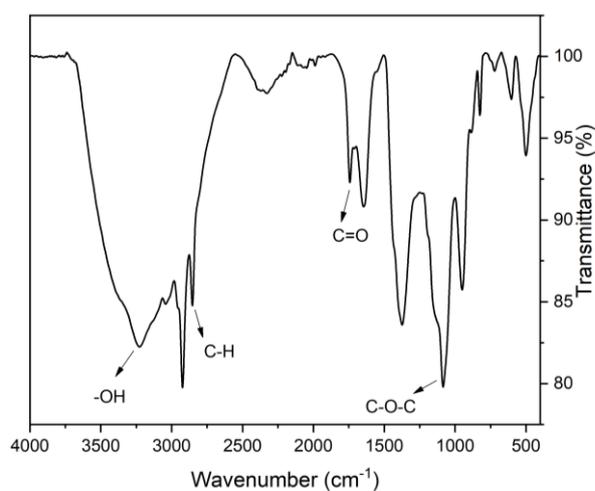
Functional group vibrations	Wavenumber (cm <sup>-1</sup> )			
	Endophytic isolate D9	Endophytic <i>Burkholderia</i> sp. WYAT7 [14]	Endophytic <i>Bacillus pumilus</i> 2A [36]	<i>Pseudomonas otitidis</i> [37]
Stretching -OH	3227.29	3005.10	3292.42	3464.15
Stretching aliphatic C-H (-CH <sub>2</sub> and -CH <sub>3</sub> )	2923.14 2853.55	2922.16 2852.72	2989.66 2893.22	2920.23 2360.87
Stretching ester C=O	1742.40	1743.65	1743	1740.08
Stretching the glycosidic bond	1084.61 885.19	1024.20 603.72	1045.92 862.03	1080.44 848.6

As illustrated in Figure 5(B), the BS-D9 biuret test did not yield negative results, as no purple coloration was observed. The BS-D9 compound lacked peptide bonds, indicating that it was excluded from the lipopeptide group. These results are consistent with those reported in the study by Albasri *et al.* [32], which indicated that biosurfactants produced by *Geobacillus stearothermophilus* exhibited negative outcomes in the biuret test. Panatula *et al.* [38] reported that biosurfactants from *Nocardiosis dassonvillei* var. B2 showed negative results, and Patel and Patel [39] stated that biosurfactants produced by fungi isolated from mangrove ecosystems also showed negative biuret test results. The reaction that occurs in the biuret test involves the formation of a coordination complex between Cu<sup>2+</sup> ions and at least two peptide bonds in the molecule. The process entails coordination of copper ions to the nitrogen atoms of peptide bonds, thereby forming a stable tetradentate complex. The formation of this complex results in a color change in the solution to purple or violet. The intensity of this color can be quantitatively measured, thereby serving as an indicator of the peptide bond content [35].

### 3.6. Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FTIR analysis was employed to characterize BS-D9, based on the functional groups present in the compound. The FTIR results for BS-D9 (Figure 6) indicate the presence of an absorption band at 3227.29 cm<sup>-1</sup>, which is indicative of -OH stretching. Additionally, the data show absorption bands between 2923.14 cm<sup>-1</sup> and 2853.55 cm<sup>-1</sup>, suggesting -CH<sub>2</sub> and -CH<sub>3</sub> stretching. The maximum peak observed at 1742.40 cm<sup>-1</sup> corresponds to the C=O stretching of the ester carbonyl group. The peaks at 1084.61 cm<sup>-1</sup> and 885.19 cm<sup>-1</sup> are associated with the stretching vibrations of the glycosidic bond.

The FTIR spectrum of BS-D9 was then compared with those of biosurfactants from microorganisms for identification. Research conducted by A. *et al.* [14] successfully identified glycolipid biosurfactants from *Burkholderia* sp. WYAT7, and research [11] identified biosurfactants from the endophytic bacterium *Bacillus pumilus* 2A. *Pseudomonas otitidis* bacteria were also identified as capable of producing glycolipids [37]. As illustrated in Table 1, a comparative analysis of the FTIR spectra of BS-D9 and the referenced materials is presented.

**Figure 6.** FTIR spectrum of BS-D9 results

BS-D9 is a glycolipid, potentially a rhamnolipid. The presence of glycosidic bonds, which are characteristic of glycolipids [36], further substantiates this claim. The D9 biosurfactant isolate that was examined contains -OH groups, aliphatic C-H, C=O ester, and glycosidic bonds in the fingerprint region. The BS-D9 demonstrates notable capacity, with an E24 value of 95%, suggesting its potential for application in the cosmetics and pharmaceutical industries as an alternative to synthetic surfactants.

Consequently, further analysis is necessary to ascertain the bioactivity, including but not limited to antioxidants, antibacterials, and anti-inflammatories. Moreover, analysis with GC-MS instruments is required to determine the specific compound name of the biosurfactant type.

## 4. Conclusion

The endophytic bacteria isolate D9 demonstrated the capacity to produce biosurfactants, with an optimal production time of 132 hours. It has been demonstrated that BS-D9 can disperse oil, with an emulsion index equivalent to SDS at 1000 ppm. This is evidenced by measurements of 5.1 cm and 95%, respectively. The methylene blue test indicated that BS-D9 is an anionic biosurfactant, and the negative biuret test result indicated that BS-D9 is not a lipopeptide. The Fourier-transform infrared (FTIR) analysis of BS-D9 revealed characteristic peaks indicative of a glycolipid

biosurfactant, with spectral features consistent with rhamnolipid-type compounds. However, prior to further industrial application, additional evaluation of the toxicity and stability of BS-D9 is required to ensure its safety and performance consistency.

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